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SHORT COMMUNICATION

AUTORADIOGRAPHIC AND CYTOCHEMICAL STUDIES ON NUCLEAR AND CYTOPLASMIC INCLUSIONS OF DUCK EMBRYO FIBROBLASTS INFECTED WITH HERPES TYPE VIRUS ISOLATED FROM CHICKENS WITH MAREK'S DISEASE[1]

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Many studies have provided strong evidence that a herpes-type virus (HTV) is the cause of Marek's disease (MD)(Churchill and Biggs, 1967; Solomon et al., 1968; Nazerian et al., 1968; Biggs et al., 1969). Three strains of the HTV were isolated in duck embryo fibroblasts (DEF) by the method of Solomon et al. (1968) (Kato et al., 1969). The biological and morphological characteristics of these strains of virus in tissue culture were nearly identical with those described by the authors mentioned above. The strains were named the Biken A, B and C strain. DEF infected with one of these strains produced typical herpes type nuclear inclusions just like those described by Churchill (1968). Besides nuclear inclusions, we also observed eosinophilic cytoplasmic inclusions in these cells, which have not been described previously.

This communication described autoradiographic and cytochemical studies on these new cytoplasmic inclusions in comparison with nuclear inclusions. Throughout the experiments the specificity of the cytopathic effects of the HTV was confirmed by the direct fluorescent antibody technique described by Naito et al. (1969).

DEF cultures were inoculated with one of the Biken strains. Typical foci developed 3–5 days after inoculation. These foci consisted of many round cells and multinucleated giant cells (Fig. 1). Using Bouin's fixative and hematoxylin-eosin staining, the cells constituting the foci were seen to have many eosinophilic cytoplasmic inclusions as well as herpes type nuclear inclusions which stained with hematoxylin (Fig. 1, 2). Cells with cytoplasmic (C) inclusions always contained nuclear (N) inclusions as well. Therefore, C inclusion formation seems to occur subsequent to N inclusion formation. C inclusions only stain with eosin and having halos around them. They are generally irregular in shape and vary in size from less than 1 μ to a few μ in diameter. The number of C inclusions varies from a few to very many per cell and sometimes all the cytoplasmic area was occupied by numerous C inclusions of various shapes. C inclusions did not stain with Schiff's polysaccharide stain. They also gave a negative Feulgen reaction, while N inclusions gave a positive reaction. These two types of inclusions in DEF inoculated with Biken's strains were detectable from the beginning of virus isolation up to the 36th passage in DEF.

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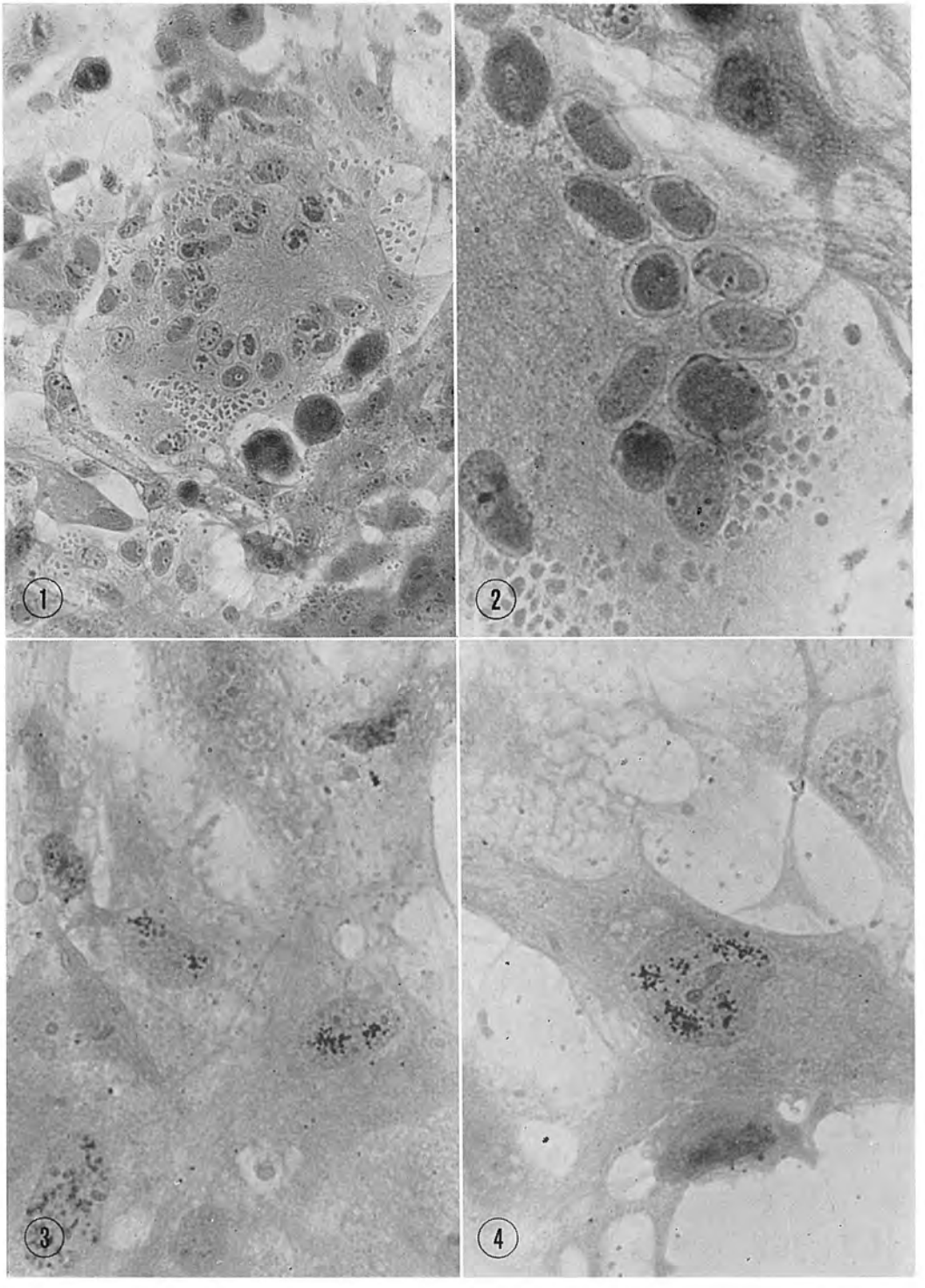
FIGURE 1. *A multinucleate giant cell in a microfocus in a monolayer culture of duck embryo fibroblasts 5 days after inoculation with duck embryo fibroblasts infected with Biken B strain. Various forms of nuclear inclusions and cytoplasmic inclusions are present. Fixed with Bouin's fluid, stained with hematoxylin-eosin.*

FIGURE 2. *High magnification of the giant cell shown in Fig. 1.*

FIGURE 3–8. *Autoradiograms of duck embryo fibroblasts 4 days after inoculation with duck embryo fibroblasts infected with Biken C strain after exposure to ^{3}H-thymidine for 1 hr. Fixed with Bouin's fluid, stained with hematoxylin-eosin. Note nuclear foci of grains, and cytoplasmic inclusions free from silver grains.*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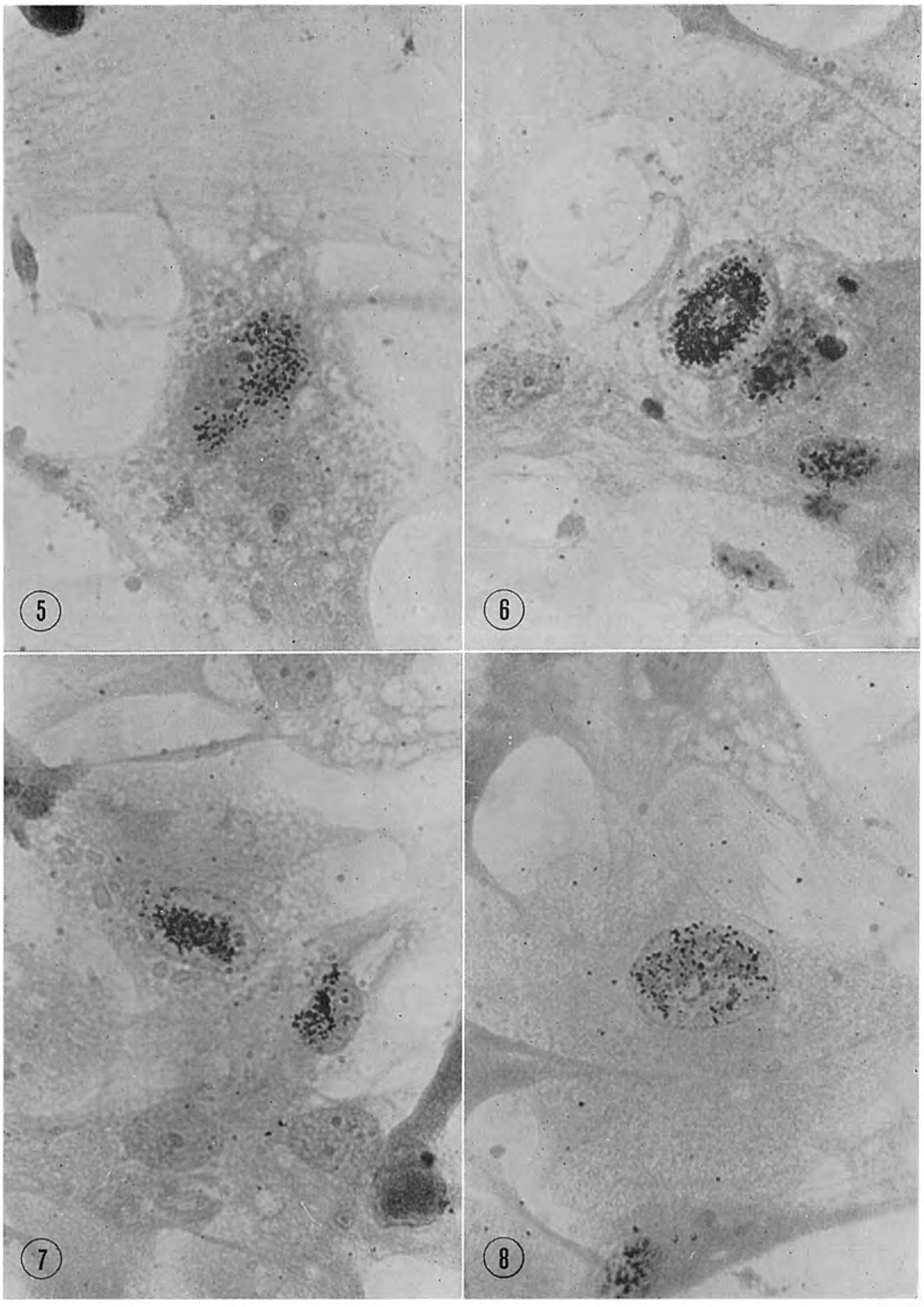
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HTV-infected DEF showing typical cytopathic effects of the HTV were kept in medium containing ^{3}H-thymidine (5 μc/ml, specific activity 5 c/mM) for 1 hr. Then the cells were fixed in Bouin's fluid for 2 hr and treated with 2% perchloric acid for 40 min at 4 C. Then autoradiography was carried out by the dipping method using Sakura NR-M2 nuclear emulsion. After 5 days exposure, the preparations were developed, fixed and stained with hematoxylin-eosin. As shown in Fig. 3–8, many cells constituting the typical focus contained irregular shaped accumulations of silver grains in the center of their nuclei. Generally the marginated chromatin and nucleolus were free from silver grains. Grains were concentrated in one or two areas of the nucleus, probably representing an early stage of infection (Fig. 3). Sometimes silver grains were aggregated in bizarre shapes (Fig. 5) or ring shapes with a nucleolus at the center (Fig. 6). In some cells the areas of silver grains were identical with those of nuclear inclusions (Figs. 7, 8). The appearance of silver grains in the nuclei of HTV-infected DEF was similar to that in the nuclei of Vero cells infected with varicella-zoster virus (Nii and Maeda, 1969).

These results show that DNA synthesis of the HTV takes place exclusively in the nuclei of infected cells. The cytoplasmic inclusions were not related to DNA synthesis. The significance of the cytoplasmic inclusions in viral multiplication is now under investigation.

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